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Clinical report

Induction of cytokines and killer cell activities by cisplatin and 5-fluorouracil in head and neck cancer patients

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It has been suggested that certain antitumor agents stimulate antitumor immunity. In the present study, we examined whether cisplatin and 5-fluorouracil (5-FU) accelerate the antitumor host responses in head and neck cancer patients. Two groups of patients were studied, i.e. an untreated (UT) group and a treated, disease-free (TDF) group that received chemo-immunotherapy in combination with radiotherapy and operation. When peripheral blood mononuclear cells (PBMC) derived from head and neck cancer patients were treated with cisplatin or with 5-FU, interferon-γ, tumor necrosis factor (TNF)-α, TNF-β, interleukin (IL)-1β, IL-6, IL-12 and IL-18 as well as killer cell activities were significantly induced in both groups. In this case, these activities induced by cisplatin in UT showed lower levels than those in TDF, whereas the activities induced by 5-FU in the UT group demonstrated almost similar levels to those in TDF. These activities were significantly inhibited by anti-asialo-GM_1 antibody. Furthermore, cytokine levels in sera and killer activities of PBMC derived from the cancer patients were significantly increased after cisplatin administration. These findings suggest that cisplatin and 5-FU increase anticancer immunity mediated by induction of cytokines and killer cell activities in patients with head and neck cancer. [© 2000 Lippincott Williams & Wilkins.]

Key words: 5-Fluorouracil, cisplatin, cytokines, head and neck cancer, killer cell activity.

Introduction

Cisplatin and 5-fluorouracil (5-FU) have been used successfully in the treatment of many types of human malignancies including head and neck cancer.[1–4] Although chemotherapeutic agents such as cisplatin and 5-FU are generally assumed to cause depression of immune responsiveness,[5] it has also been reported that certain antitumor agents are actually stimulatory to host responses. Cisplatin is reported to enhance the activities of monocytes and natural killer (NK) cells *in vivo* and *in vitro*[6–8] to induce lymphokine-activated killer (LAK)-like cells in combination with methotrexate, vinblastine and adriamycin in bladder cancer patients,[9] and to enhance interleukin (IL)-2 synthesis by human primary blood lymphocytes.[10] Although there are only few reports regarding the increasing effects of immune response by 5-FU, it has been demonstrated that 5-FU augments mouse LAK cell activity induced by IL-2.[11]

Additionally, we have reported that the treatment of peripheral blood mononuclear cells (PBMC) derived from healthy volunteers with cisplatin or with 5-FU markedly induced NK and LAK activities as well as several cytokines such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, (TNF)-β, interleukin (IL)-1β, IL-6 and IL-12 mediated by asialo-GM_1-positive cells as well as T cells, and that these activities are closely associated with the *in vivo* antitumor effect of these therapeutic agents both in the salivary gland tumor-bearing nude mice model and in the syngeneic Meth-A tumor-bearing BALB/c mice model.[12]

It is important to identify chemotherapeutic agents which increase host immune response because these effects may be associated with the antitumor efficiency exhibited by the treatment with the agents, and

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because the agents would be significant candidates for use in combined chemo-immunotherapy protocols. In the present study, we examined whether cisplatin and 5-FU may also increase antitumor immunity including cytokine production, and NK and LAK activities in patients with head and neck cancer.

Materials and methods

Patients

Twenty-three patients with head and neck cancer were divided into two groups, i.e. 11 patients in an untreated group (UT) and 12 patients in a treated, disease-free group (TDF) in which no recurrent tumors had been observed for 1 year or more. The simultaneous combination therapy with external radiation (^{60}Co; a total dose of 50–60 Gy), UFT (an oral preparation combining the 5-FU pro-drug tegafur and uracil in a 1:4 ratio; 200–400 mg/day for 4–6 weeks) and OK-432 (a streptococcal preparation for immunotherapy; 5 KE=0.5 mg/week for 4–6 weeks) was performed as a primary therapy in all of the patients in TDF. After the primary therapy, residual tumors were surgically removed in five of 12 patients in TDF. PBMC were collected from the patients by the standard Ficoll-Hypaque gradient-density centrifugation method.[13]

Treatment of PBMC with cisplatin or with 5-FU

PBMC (1×10^6/ml) were cultured in RPMI 1640 medium containing 10% FCS in the presence or absence of cisplatin (1×10^{-3} to 1.0 μg/ml) or 5-FU (0.1–5.0 μg/ml) for 0–72 h at 37°C. Cytokines in the supernatants of these cultures were measured, and the PBMC treated with cisplatin or 5-FU were assayed for NK and LAK activities.

Cytokine assay

The IFN assay of the samples was performed by the plaque-reduction assay with vesicular stomatitis virus in FL cells as described previously.[14] The IFN titer was determined as the highest sample dilution indicating 50% reduction of plaque counts in the monolayer treated with IFN sample as compared with the untreated controls. To determine the type of IFN induced by cisplatin or 5-FU, neutralization of IFN in the samples was performed using rabbit antiserum to human IFN-α (Lee Biomolecular Research, San Diego, CA), rabbit antiserum to human IFN-β (Lee Biomolecular Research) and rabbit antiserum to human IFN-γ (Endogen, Boston, MA) as described previously.[15] The amount of IFN-γ in the sera derived from the patients treated with cisplatin was measured by an ELISA kit (R&D Systems, Minneapolis, MN).

The TNF titer was determined as cytolytic activity against L-929 cells following the method of Ruff and Gifford.[16] The TNF titer was expressed as the highest sample dilution indicating 50% reduction of the numbers of L-929 cells treated with TNF sample as compared with the untreated controls. The determination of the type of TNF was also performed using neutralizing antibodies, such as anti-human TNF-α antibody (R&D Systems) or anti-human TNF-β antibody (R&D Systems).

The assay for other cytokines was performed using ELISA kits. The ELISA kits for IL-1β and IL-6 were purchased from Genzyme (Cambridge, MA), and the kits for IL-2, IL-4, IL-10, IL-12, IL-13 and IL-15 from BioSource International (Camarillo, CA). The ELISA system for human IL-18 using a polyclonal goat anti-human IL-18 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) as a solid-phase antibody and a monoclonal mouse anti-human IL-18 antibody (R&D Systems) as a secondary antibody was established in our laboratory. The assay detected human IL-18 at 10 pg/ml or higher in a linear fashion.

Assay for NK and LAK cell activities

The cytotoxic activities of human PBMC were assayed against K-562, a markedly sensitive target for human NK cells, and Daudi, a sensitive target for human LAK cells but not destroyed by human NK cells, in a ^{51}Cr-release test. The ^{51}Cr release was carried out as described previously.[17] For cell-mediated cytotoxicity assays, 4.0×10^5 effector cells were mixed in the wells of 96-well microtiter plates (Falcon; Becton Dickinson Laboware, Lincoln Park, NJ) with 1.0×10^4 ^{51}Cr-labeled target cells (effector cell:target cell=40:1) in a total volume of 200 ml medium and incubated at 37°C for 4 h. The percent specific ^{51}Cr-release was calculated according to the formula: $[(E-S)/(M-S)] \times 100$, where E is experimental ^{51}Cr release, S is spontaneous ^{51}Cr release and M is maximum ^{51}Cr release. The percent specific ^{51}Cr release was considered as the NK or LAK cell activity.

Treatment of PBMC with anti-asialo-GM_1 antibody or anti-Leu4 antibody

PBMC were treated with an anti-asialo-GM_1 antibody (1:20 diluted; Wako Pure Chemical, Osaka, Japan) or anti-Leu4 antibody (1:20 diluted; Becton Dickinson, San Jose, CA) for 40 min at 4°C and then incubated

with rabbit serum complement (1:10 diluted) for 20 min at 37°C. The PBMC were cultured in the presence of cisplatin (0.1 μg/ml) or 5-FU (1.0 μg/ml), and were then assayed for IFN, TNF, IL-1β, IL-6, IL-12, IL-18, NK and LAK activities.

Cytokine levels in sera, and NK and LAK activities of PBMC derived from cisplatin-treated head and neck cancer patients

Five patients with head and neck cancer were treated i.v. with cisplatin (45–100 mg/m^2). Both 72 h before and 24 h after cisplatin treatment, the sera and the PBMC were collected for examination of cytokines, and NK and LAK activities, respectively. For measuring pre-LAK activity, the PBMC derived from the patients given cisplatin were cultured with 1000 IU/ml of recombinant IL-2 for 72 h before the analysis as described by Aramaki *et al.*[18] The PBMC were also analyzed for the surface markers of lymphocytes using specific monoclonal antibodies and a fluorescence-activated cell sorter (FACS), CS-20S (Showa Denko, Tokyo, Japan).

Statistical analysis

The data were evaluated by the Mann–Whitney *U*-test. $p < 0.05$ was considered significant.

Results

Induction of cytokines, and NK and LAK activities by cisplatin and 5-FU on PBMC isolated from head and neck cancer patients

Cisplatin (1×10^{-3} to 1.0 μg/ml) or 5-FU (0.1–5.0 μg/ml) was added into the PBMC culture derived from head and neck cancer patients including the UT group and TDF group. Twenty-four hours later, cytokines in the supernatants, and NK and LAK activities of the PBMC were analyzed. In both groups, the treatment of the PBMC with cisplatin significantly increased NK and LAK activities as well as the production of IFN, TNF, IL-1β, IL-6, IL-12 and IL-18 as compared with respective untreated controls. However, these activities, except IL-6, of PBMC from the patients in the UT group showed lower levels than those in the TDF group, especially IFN- and IL-18-producing activities (Figure 1). These cytokine- and killer cell-inducing activities of cisplatin in the TDF group demonstrated almost the same levels as those in healthy volunteers[12] (unpublished observations). No induction of IL-2, IL-4, IL-10, IL-13 and IL-15 was observed in any group (data not shown). In contrast, 5-FU-treated PBMC from the patients in the UT group demonstrated similar levels to those from the TDF group in NK and LAK activities (Figure 2A). 5-FU also induced many types of cytokines such as IFN, TNF, IL-1β, IL-6, IL-12 and IL-18 to a larger degree than respective untreated controls (Figure 2C–G). The increase of production of IL-2, IL-4, IL-10, IL-13 and IL-15 was not shown by 5-FU treatment (data not shown). All of the activities tested showed maximum levels at 24 h after cisplatin or 5-FU stimulation, and subsequently they gradually decreased (data not shown). The IFN activity induced by cisplatin or 5-FU in the patients in both groups was almost completely neutralized by anti-human IFN-γ serum but not by anti-human IFN-α/β serum (data not shown). Therefore, most of the IFN induced by these agents was determined as IFN-γ. In addition, both TNF-α and TNF-β were induced by PBMC treated with cisplatin or 5-FU in the TDF group as well as the UT group (data not shown).

Effect of an anti-asialo-GM_1 or an anti-Leu4 antibody on cytokine-, and NK- and LAK-inducing activities of cisplatin or of 5-FU in head and neck cancer patients

To clarify the subpopulations which elicit cytokine-, NK- and LAK-inducing activities after treatment with cisplatin or 5-FU, PBMC isolated from the patients in the UT group were treated with an anti-asialo-GM_1 or with an anti-Leu4 antibody in the presence of rabbit serum complement before stimulation of the agents. All of the activities induced by cisplatin or 5-FU were markedly inhibited by treatment with anti-asialo-GM_1 antibody and complement. LAK activity induced by cisplatin as well as TNF, IL-12 and LAK activity induced by 5-FU stimulation were also significantly inhibited by anti-Leu4 antibody and complement (Tables 1 and 2). Similar results were observed the in TDF group (data not shown).

Induction of cytokines, and NK and LAK activities by cisplatin administration in patients with head and neck cancer

Twenty-four hours after cisplatin treatment in head and neck cancer patients, the sera' and the PBMC were collected, and cytokines in the sera and NK and LAK activities of the PBMC were analyzed. NK and LAK activities were increased after cisplatin administration in four of five head and neck cancer patients (Figures 3A and B), whereas pre-LAK activity was enhanced only in one of five patients (Figure 3C). The amounts of IFN-γ were increased in five of five patients (Figure 3D), TNF-α in four of five (Figure 3E), TNF-β in

four of five (Figure 3F), IL-1β in four of five (Figure 3G), IL-6 in three of five (Figure 3H), IL-10 in two of five (Figure 3I), IL-12 in four of five (Figure 3J) and IL-18 in five of five (Figure 3K). IL-2, IL-4, IL-13 and IL-15

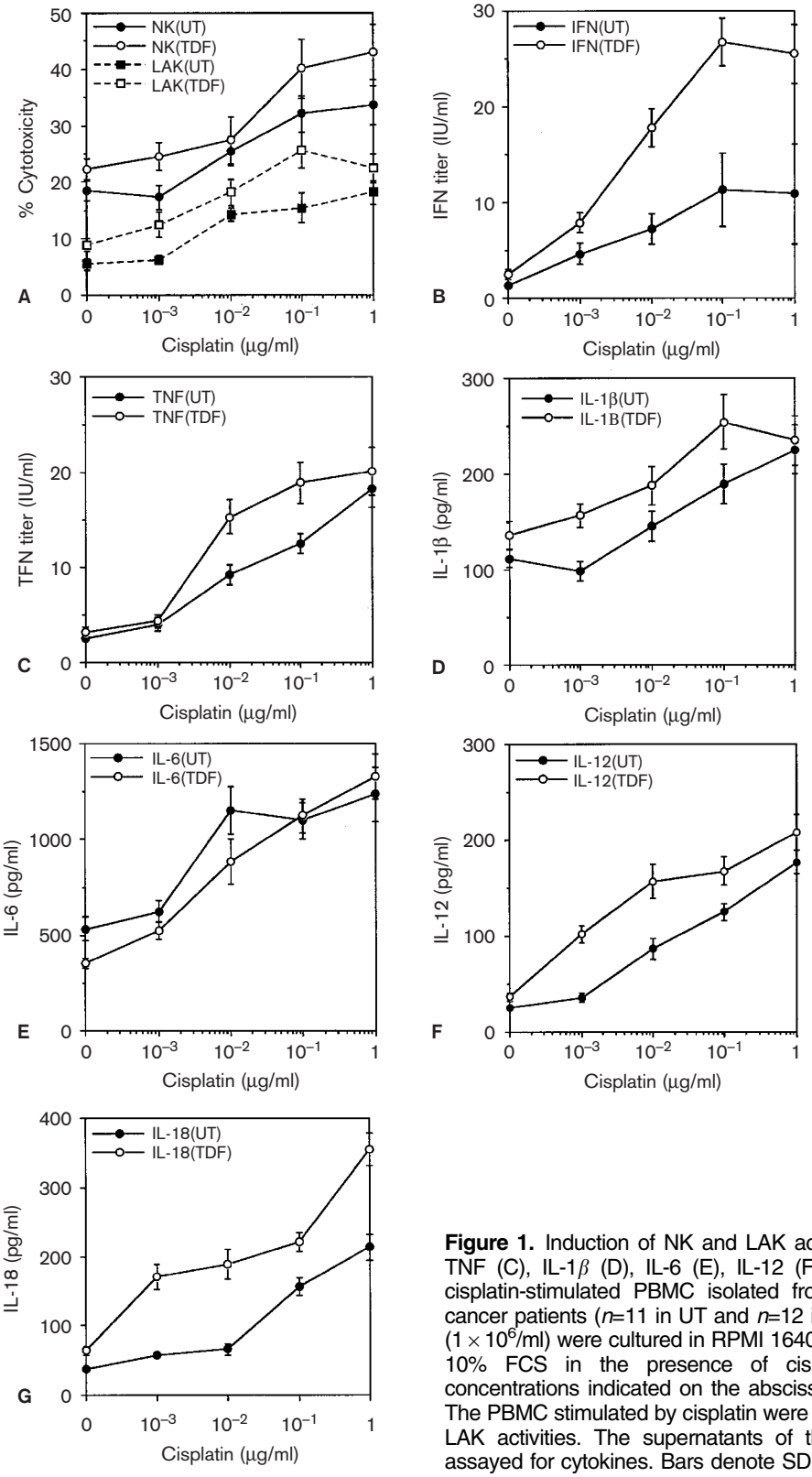

Figure 1. Induction of NK and LAK activities (A), IFN (B), TNF (C), IL-1β (D), IL-6 (E), IL-12 (F), and IL-18 (G) in cisplatin-stimulated PBMC isolated from head and neck cancer patients (n=11 in UT and n=12 in TDF). The PBMC (1×10^6/ml) were cultured in RPMI 1640 medium containing 10% FCS in the presence of cisplatin at the final concentrations indicated on the abscissa for 24 h at 37°C. The PBMC stimulated by cisplatin were assayed for NK and LAK activities. The supernatants of these cultures were assayed for cytokines. Bars denote SD.

were not detected in the sera from all of five patients analyzed (data not shown). PBMC were also analyzed for the surface markers of lymphocytes using specific monoclonal antibodies as shown in Table 3. No

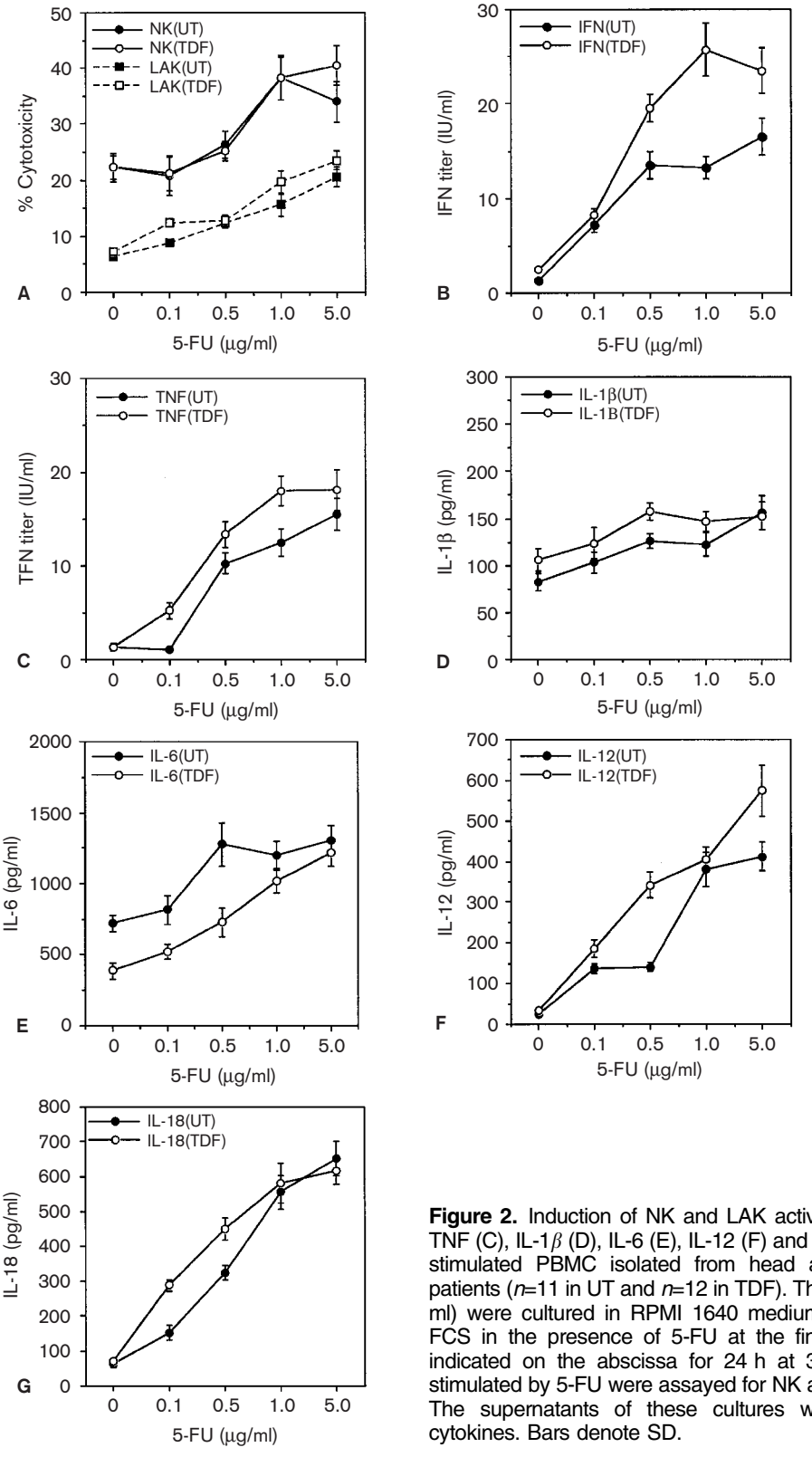

Figure 2. Induction of NK and LAK activities (A), IFN (B), TNF (C), IL-1β (D), IL-6 (E), IL-12 (F) and IL-18 (G) in 5-FU-stimulated PBMC isolated from head and neck cancer patients (n=11 in UT and n=12 in TDF). The PBMC (1×10^6/ml) were cultured in RPMI 1640 medium containing 10% FCS in the presence of 5-FU at the final concentrations indicated on the abscissa for 24 h at 37°C. The PBMC stimulated by 5-FU were assayed for NK and LAK activities. The supernatants of these cultures were assayed for cytokines. Bars denote SD.

Table 1. Effect of an anti-asialo-GM_1 or an anti-Leu4 antibody on cytokine-, NK- and LAK-inducing activities of cisplatin

Treatment	IFN (IU/ml)	TNF (IU/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-12 (pg/ml)	IL-18 (pg/ml)	NK activity (%)	LAK activity (%)
Untreated	2.8±0.5	3.5±0.8	108±19	554±78	19.7±3.3	32.2±6.5	21.3±3.5	4.1±0.3
Cisplatin alone	14.3±2.2	15.2±3.6	226±42	1287±177	211±13	182±11	36.2±5.3	20.8±3.7
Cisplatin + anti-asialo-GM_1 + C	3.3±0.6[a]	6.7±1.6[a]	117±17[a]	695±89[a]	86±9.0[a]	89.7±11.3[a]	18.4±3.3[a]	12.5±3.2[b]
Cisplatin + anti-Leu4 + C	12.6±1.7	13.7±0.4	195±35	1164±11	187±11	173±13	34.4±2.8	9.1±1.6[a]
Cisplatin + C	15.5±3.0	16.8±3.4	251±45	1229±135	195±23	204±14	39.1±4.5	22.7±4.0

PBMC from head and neck cancer patients in the UT group were treated with an anti-asialo-GM_1 or an anti-Leu4 antibody (1:20 diluted) for 40 min at 4°C and then incubated with rabbit serum complements (1:10 diluted) for 20 min at 37°C. The PBMC were cultured in the presence of cisplatin (0.1 μg/ml), and were then assayed for IFN, TNF, IL-1β, IL-6, IL-12, IL-18, NK and LAK activities. Results are expressed as mean ± SD (n=11).
[a]$p<0.001$, [b]$p<0.05$ as compared with respective positive controls treated with cisplatin alone.

Table 2. Effect of an anti-asialo-GM_1 or an anti-Leu4 antibody on cytokine-, NK- and LAK-inducing activities of 5-FU

Treatment	IFN (IU/ml)	TNF (IU/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-12 (pg/ml)	IL-18 (pg/ml)	NK activity (%)	LAK activity (%)
Untreated	2.6±0.3	5.5±1.0	97±23	476±61	22.7±3.3	43.5±3.5	18.2±2.0	5.7±1.2
5-FU alone	29.3±3.6	18.7±2.5	234±34	865±101	462±55	582±62	41.1±6.4	19.5±4.2
5-FU + anti-asialo-GM_1 + C	15.8±2.2[a]	10.1±2.0[a]	168±20[a]	574±62[a]	196±37[a]	225±35[a]	15.5±4.7[a]	9.6±1.6[a]
5-FU + anti-Leu4 + C	25.1±3.1	14.2±0.8[b]	218±32	824±114	372±19[b]	540±28	38.9±5.6	12.9±2.7[b]
5-FU + C	32.3±5.6	22.3±2.7	244±28	853±87	507±66	38.8±5.5	40.5±4.5	21.2±5.4

PBMC from head and neck cancer patients in the UT group were treated with an anti-asialo-GM_1 or an anti-Leu4 antibody (1:20 diluted) for 40 min at 4°C and then incubated with rabbit serum complements (1:10 diluted) for 20 min at 37°C. The PBMC were cultured in the presence of 5-FU (1.0 μg/ml), and were then assayed for IFN, TNF, IL-1β, IL-6, IL-12, IL-18, NK and LAK activities. Results are expressed as mean ± SD (n=11).
[a]$p<0.001$, [b]$p<0.05$ as compared with respective positive controls treated with 5-FU alone.

Table 3. Surface markers of the lymphocytes derived from head and neck cancer patients administered cisplatin

	Before	After
WBC (/mm^3)	6550±2345	3350±732[a]
Lymphocyte (/mm^3)	2267±929	1141±379[a]
$CD3^+$ (%)	62.7±14.2	68.1±15.9
$CD4^+$ (%)	36.4±9.3	40.7±12.6
$CD8^+$ (%)	29.8±9.3	27.1±12.1
$CD16^+$ (%)	19.3±10.9	15.5±12.1
$CD57^+$ (%)	23.6±4.7	21.2±12.1
$CD3^+HLA\text{-}DR^+$ (%)	11.1±8.7	10.7±4.4
$CD4^+CD45^-$ (%)	11.3±2.6	13.6±1.8
$CD4^+CD45^+$ (%)	24.9±7.5	29.3±14.7
$CD8^+CD11^-$ (%)	18.0±4.5	16.4±2.4
$CD8^+CD11^+$ (%)	6.4±0.8	7.7±7.0
$CD16^+CD57^-$ (%)	15.4±6.4	15.5±3.0
$CD16^-CD57^+$ (%)	3.9±1.7	3.3±3.4
$CD16^+CD57^+$ (%)	14.2±3.0	12.1±11.5

Five patients with head and neck cancer were treated i.v. with cisplatin (45–100 mg/m^2). Both 72 h before and 24 h after cisplatin treatment, the PBMC were collected and were analyzed for the surface markers of lymphocytes using specific monoclonal antibodies and FACS.
[a]$p<0.05$; as compared with respective controls before cisplatin administration.

significant difference was observed between before and after cisplatin administration in all of the surface markers tested.

Discussion

Cisplatin and 5-FU have been used successfully in the treatment of many types of human malignancies including head and neck cancer.[1–4] Furthermore, these anticancer agents stimulate antitumor host responses in patients with malignant deseases;[6–11] these effects are due to elimination of suppressor cells or due to stimulation of killer cells.[19] Recently, we have reported that the treatment of PBMC isolated from healthy volunteers by cisplatin or by 5-FU markedly induced several cytokines such as IFN-γ, TNF-α, TNF-β, IL-1β, IL-6 and IL-12, as well as NK and LAK activities mediated by asialo-GM_1-positive cells, and that these accelerated host responses are significantly involved in the *in vivo* antitumor effect of these therapeutic agents both in salivary gland tumor-bearing nude mice and in syngeneic Meth-A tumor-bearing BALB/c mice.[12]

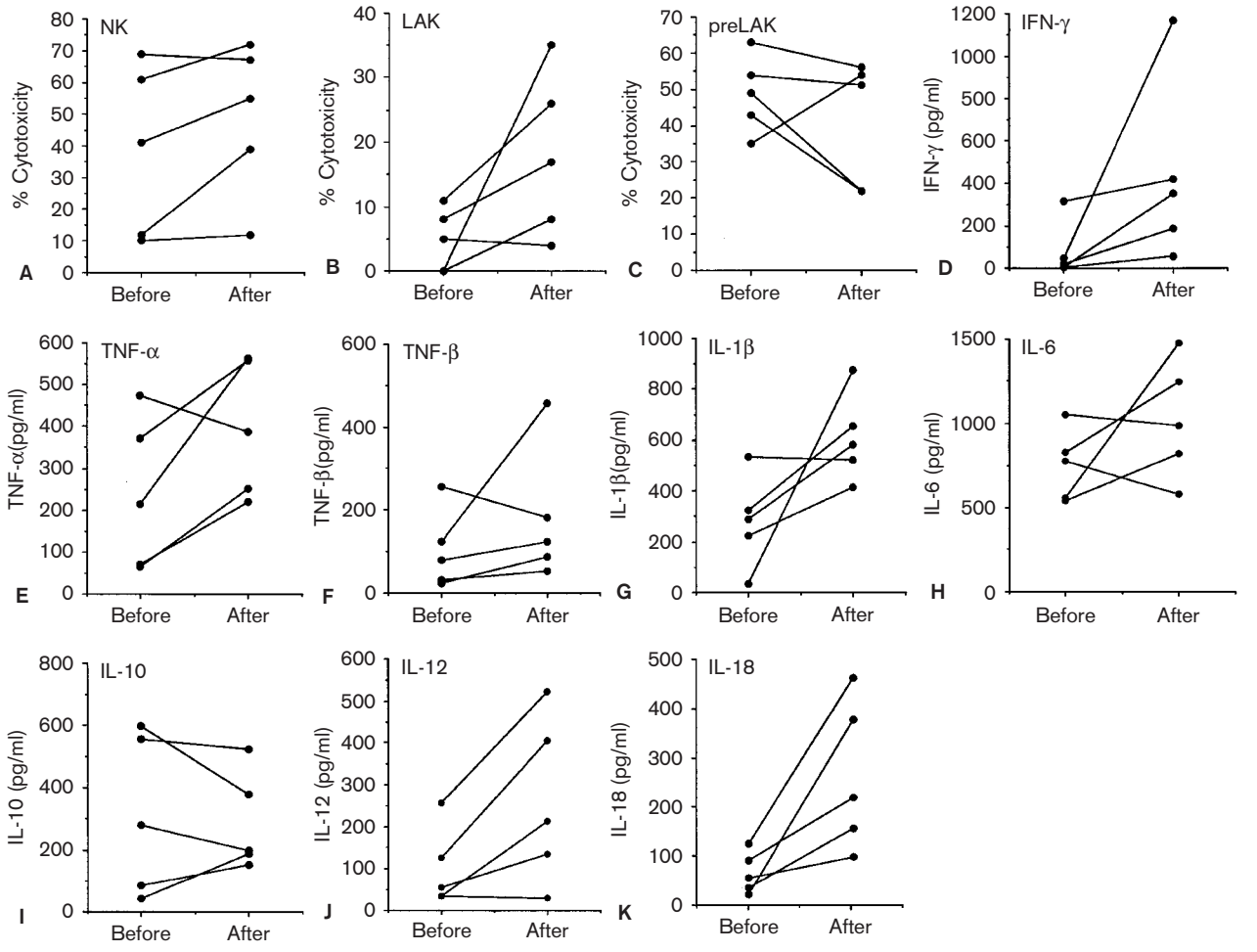

Figure 3. Five patients with head and neck cancer were administered i.v. with cisplatin (45–100 mg/m^2). Both 72 h before and 24 h after cisplatin treatment, the sera and the PBMC were collected. NK (A), LAK (B) and pre-LAK (C) activities of the PBMC, and IFN-γ (D), TNF-α (E), TNF-β (F), IL-1β (G), IL-6 (H), IL-10 (I), IL-12 (J) and IL-18(K) in the sera were examined as described in Materials and methods.

In the present study, using PBMC and sera derived from the patients with head and neck cancer in two groups, i.e. the UT and TDF group, the inducing abilities of cytokines and killer cells carrying antitumor activity of cisplatin and 5-FU were examined. The results observed in the *in vitro* experiments demonstrated that the treatment of cisplatin or 5-FU actually accelerated cytokine production, and NK and LAK activities on PBMC isolated from head and neck cancer patients. In this case, these activities induced by 5-FU in the UT group showed almost similar levels to those in the TDF group as well as healthy donors, whereas the activities induced by cisplatin in the UT group demonstrated lower levels than those in the TDF group. Furthermore, 5-FU stimulation induced large amounts of IFN-γ, IL-12 and IL-18 as compared with cisplatin treatment on PBMC from cancer patients. The results are consistent with our previous observations using PBMC from healthy donors[12] (unpublished observations). IL-12 and IL-18 as well as IFN-γ are known to be significant cytokines for anticancer immunity. IL-12 was recently reported to show a significant antitumor effect which is due to induction of T_h1 cytokines (especially IFN-γ) as well as to its antiangiogenic activity.[20,21] IL-18 was originally identified as an IFN-γ-inducing factor (IGIF) in mice with endotoxin shock[22] and the recombinant cytokine induced the production of T_h1 but not of T_h2 cytokines.[23,24] Furthermore, it was reported that IL-18 also induces IFN-γ on NK cells.[25] It is suggested that 5-FU has an advantage over cisplatin in IL-12- and IL-18-inducing ability in cancer patients, and that it is important in the antitumor activity of 5-FU.

Furthermore, we also investigated cytokine produc-

tion, and NK and LAK activities in head and neck cancer patients which were given cisplatin. NK and LAK activities were increased after cisplatin administration in most of the patients tested, whereas pre-LAK activity was not enhanced in the patients given cisplatin. This suggests that cisplatin plays a significant role during the maturation of LAK cells but not induction of LAK precursor cells. This is strongly supported by reports demonstrating that cisplatin accelarated NK and LAK activities induced by IL-2 or by IFN-γ.[6,7] In sera from cisplatin-treated patients, cytokine levels of IFN-γ, TNF-α, TNF-β, IL-1β, IL-12 and IL-18 were increased in most of the patients tested. Therefore, it appears that cisplatin administration accelerates the production of T_h1-type cytokines in patients with head and neck cancer. In fact, the patients that received cisplatin and subsequently showed increased levels of killer cell activities and T_h1 type cytokines, demonstrated better prognosis than the other patients which showed no immune response to cisplatin (data not shown). Both *in vitro* and in patients, these anticancer agents did not induce IL-4, IL-10 and IL-13. Since these cytokines are suggested to inhibit antitumor host responses,[26,27] loss of IL-4-, IL-10- and IL-13-inducing ability of the agents is also significant in the treatment of cancer patients. In addition, although cytokine production and killer celll activities were increased in patients after cisplatin administration, no significant difference in the ratio of lymphocyte-specific markers, such as CD3, CD4, CD8, CD16 and CD57, was observed between before and after cisplatin treatment. Numbers of NK cells and several subpopulations of T cells after cisplatin administration were rather decreased than before cisplatin injection in terms of absolute values. Therefore, cisplatin appears to enhance the killer cell and cytokine-producing activities in effector cells such as NK or T cells, but dose not increase the number of effector cells.

5-FU is frequently used as one of the agents for combination therapy with radiation and immunomodulators (mainly streptococcal preparation OK-432) in our clinic, and the combination therapy is more effective in the treatment for head and neck cancer patients than single therapy.[3] Since there were few cases in which only 5-FU was used for treatment, the dynamic changes of cytokine production and killer cell activities between before and after administration of 5-FU alone was not analyzed in this study. Recently, it was suggested that the cytokine balance before and after cancer treatment is a significant factor in the prognosis of cancer patients, and that a selective induction of T_h1-type cytokines, such as IFN-γ, TNF, IL-2, IL-12 and IL-18, increases antitumor immune responses and causes better prognosis than that of T_h2-type cytokines, IL-4, IL-5, IL-6, IL-10 and IL-13.[28,29] The present results strongly suggest the possibility that T_h1-type cytokines, such as IFN-γ, TNF, IL-12 and IL-18, are dominant over T_h2-type cytokines in the cancer patients treated with 5-FU or cisplatin.

Actually, 5-FU and cisplatin induced several T_h1-type cytokines and killer cell activities carrying antitumor effects in patients with head and neck cancer. The findings of our studies may be significant for the establishment of new protocols of chemo-immunotherapy using cisplatin or 5-FU to enhance host immune responses against cancer cells.

Conclusions

We examined whether cisplatin and 5-FU may accelerate the antitumor immunity in head and neck cancer patients.

(i) Both cisplatin and 5-FU significantly induced IFN-γ, TNF-α, TNF-β, IL-1β, IL-6, IL-12 and IL-18 as well as NK and LAK activities on PBMC derived from head and neck cancer patients. These activities of cisplatin in the UT group showed lower levels than those in the TDF group, whereas the activities of 5-FU in the UT group demonstrated almost similar levels to those in the TDF group. IL-2, IL-4, IL-10, IL-13 and IL-15 were not induced by cisplatin and 5-FU in either groups.

(ii) All of the activities increased by cisplatin or by 5-FU were significantly inhibited by anti-asialo-GM_1 antibody and complement. LAK activity induced by cisplatin as well as TNF, IL-12 and LAK activity induced by 5-FU were also significantly but slightly reduced by anti-Leu4 antibody and complement.

(iii) Cytokine amounts (especially T_h1-type cytokines, i.e. IFN-γ, TNF-α, IL-12 and IL-18) in sera as well as NK and LAK activities of PBMC were significantly increased after cisplatin administration in most of the patiants examined.

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